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N63-15250
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3000 ARLINGTON BOULEVARD FALLS CHURCH, VIRGINIA

Research on Detection of Extraterrestrial Life by Ultraviolet Spectrophotometry

UNPUBLISHED PRELIMINARY DATA

First Quarterly Progress Report

Period covered: 1 January 1963 through 31 March 1963

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Contract No. NASw-571

Prepared for

National Aeronautics and Space Administration
Washington 25, D.C.

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

The work described in this report is directed toward the study of absorption of a narrow region of the far ultraviolet by materials of biological origin. It is based on reported observations[1,2,3] that the peptide band exhibits a characteristic absorption of ultraviolet light of 1850 to 1900 angstrom units.

METHODS

The wavelength region of interest in this program is in the vacuum ultraviolet, i.e., the region where oxygen absorbs strongly, as shown in figure 1. This interference by oxygen precludes the study of this region under normal atmospheric conditions, and establishes the requirement for the elimination of oxygen from the system. This is normally done by working under vacuum conditions. Because the region of interest is above the absorption region of nitrogen, however, oxygen may be bled out with nitrogen. This procedure proved to be completely satisfactory for excluding oxygen.

A difficulty in this study appears in the choice of optics. The use of quartz for transmission of ultraviolet light is routine, but it must be noted that not all quartz is satisfactory for transmission of ultraviolet below 2000Å. The curve shown in figure 2* indicates that the absorption of ultraviolet by quartz begins to rise sharply as the wavelength is reduced

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*H. Bomke, Vacumspektros Kople, Leipzig: J. A. Barth, 1937.

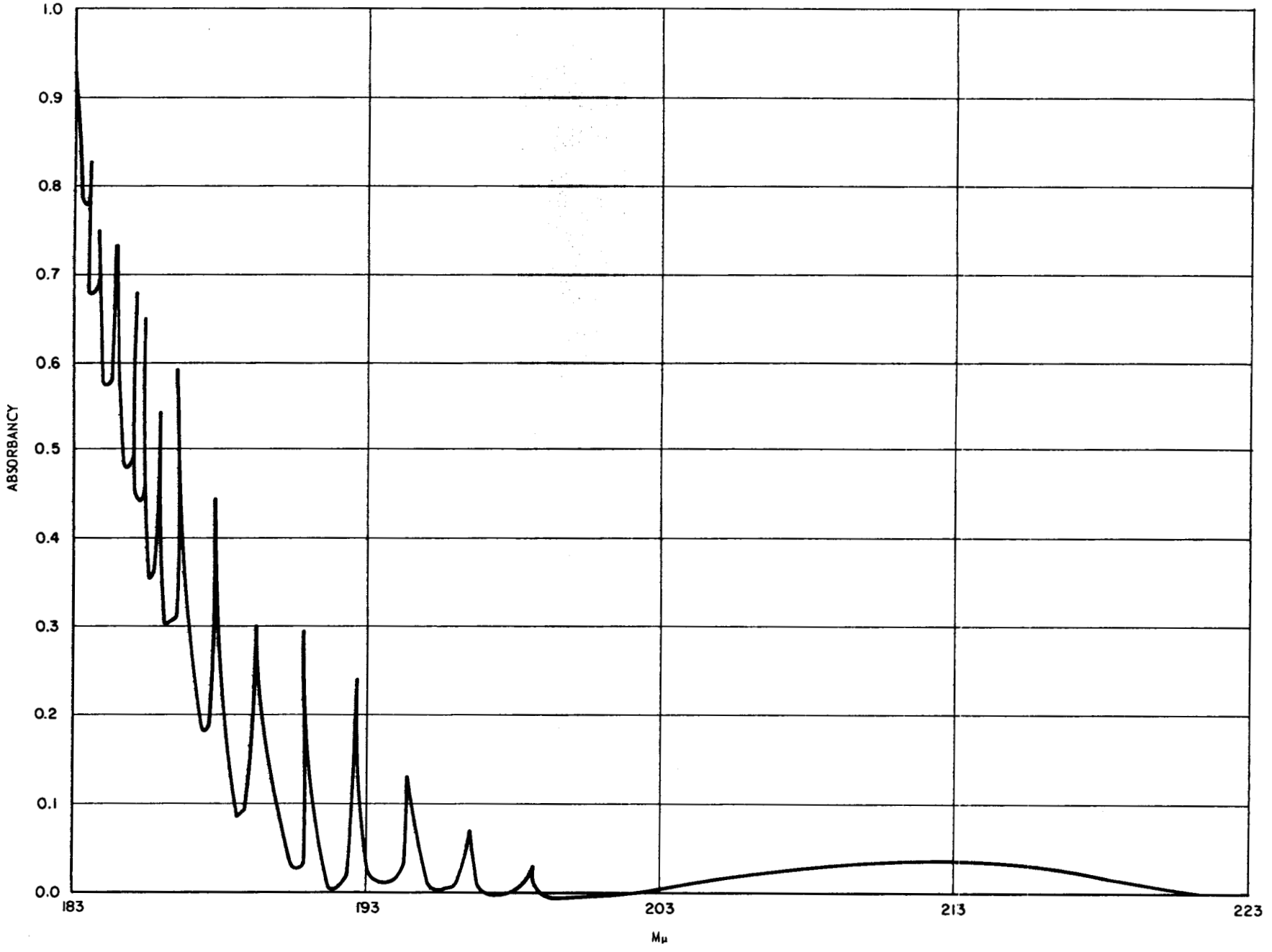

Figure 1. Absorption Spectrum of Oxygen

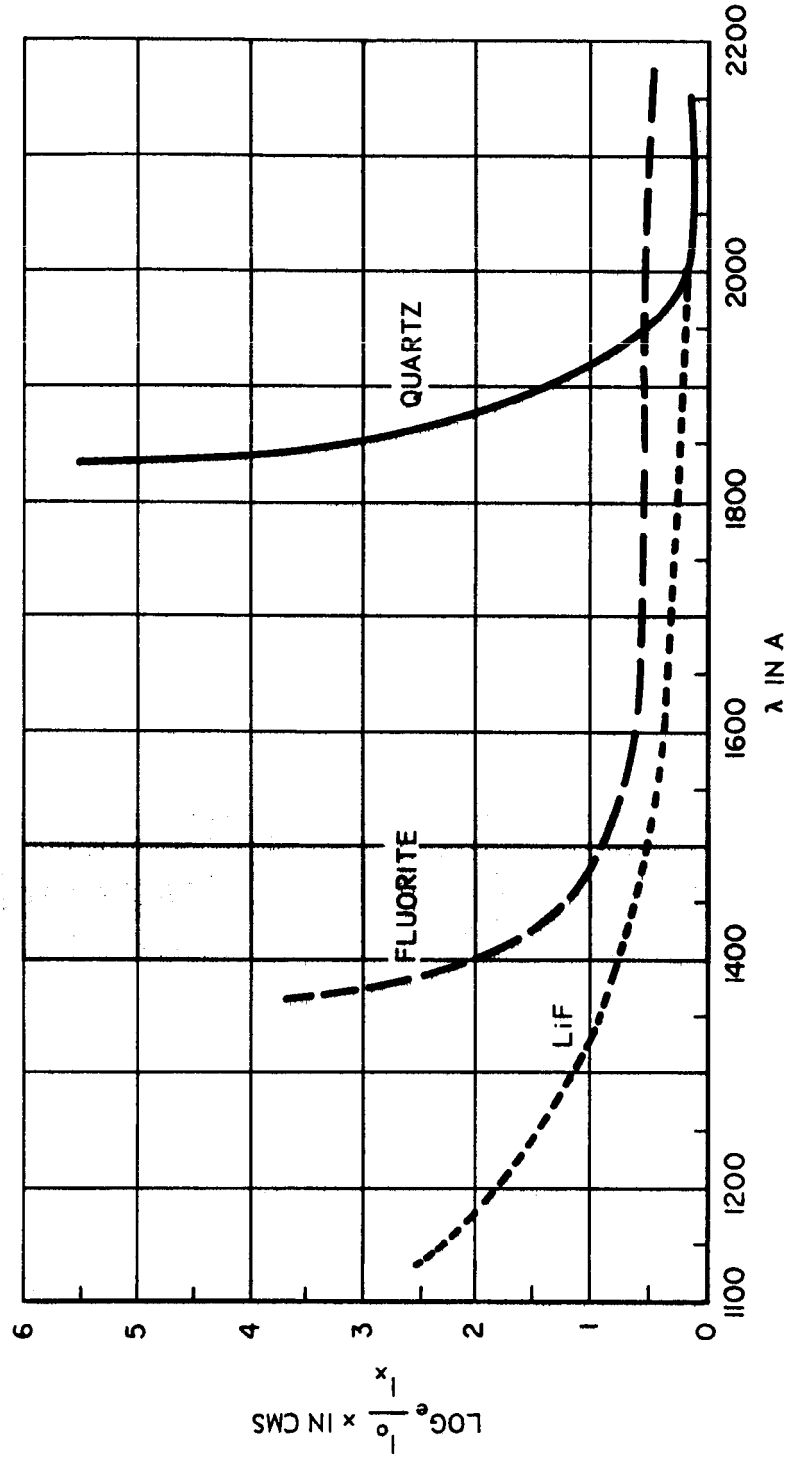

Figure 2. Absorption of U. V. by Various Materials

below 2000Å. This limitation may be overcome to some extent by the use of specially selected quartz, such as that found in the Cary 15, dual-beam spectrophotometer and similar instruments.

Associated Spectra

Spectra of the 1" x 1" x 3/64" quartz plates were obtained vs. N_2 blank. Transmission to 170 mm was observed. Thin cells were prepared with the sample and the reference representing an air span. It was observed that meaningful observations may be obtained to 180 mm before the slit function is greater than 1.0 mm. (See figure 4.)

The water cutoff at 183 is observed with 1 cm cuvettes filled with redistilled water.

Experimental Methodology

Solutions of pure materials were made with basic permanganate distilled water. Spectra were obtained by three basically different methods.

Method a. Material was disolved in concentrated glacial acetic acid and dried on quartz plates. Plates were stored under positive nitrogen pressure in a dust-free box.

Method b. Concentrated solutions of amino acids on proteins were made in redistilled water. The solution was placed between two quartz plates in such a manner as to prevent air bubbles. The thin cell was then sealed with hot paraffin wax by immersing only the edges. Copious quantities of water were used to rinse the surfaces. No evidence for deterioration of the solution within the cell was noticed over a 6-hour period.

Method c. Concentrated solutions of amino acids and proteins were studied by the use of cell inserts, which allow

a decrease in path lengths of light to 0.10 and 0.05 cm. Less concentrated solutions were analyzed by direct reading of the 1.00-cm path cell.

Instrumentation

The Cary 15 spectrophotometer was adjusted for maximum sensitivity by setting the sensitivity potentiometer at its maximum value and setting the slit to one-fourth of its width at minimal value. The dynode voltage was adjusted to its maximal position, thus reducing the slit to one thirty-second of its maximal value. Slit function was recorded with regard to wavelength for values in excess of 0.10 mm. Neutral density filters were used only when there was no other way to obtain values. Optical densities were recorded on the 0 - 20 D unit scales.

Prepurified nitrogen was flushed through the monochromations for at least one hour prior to analysis at a rate of 0.4 cu ft/minute to sweep residual O_2 and H_2O from the monochromator and all compartments. A nitrogen flow of 0.2 cu ft per minute is used during analysis. Nitrogen flushing was not required above 198 mμ.

A deuterium lamp was used during one phase of this work; after a total of less than 50 hours, however, the energy output had dropped to 0 for the energy region below 190 mm.

Calibration

The monochromator was calibrated against benzene vapors (redistilled over anhydrous calcium sulfate) and corrections were applied to the spectra. Temperature correction appears not to be a major problem. A typical calibration curve is shown in figure 3.

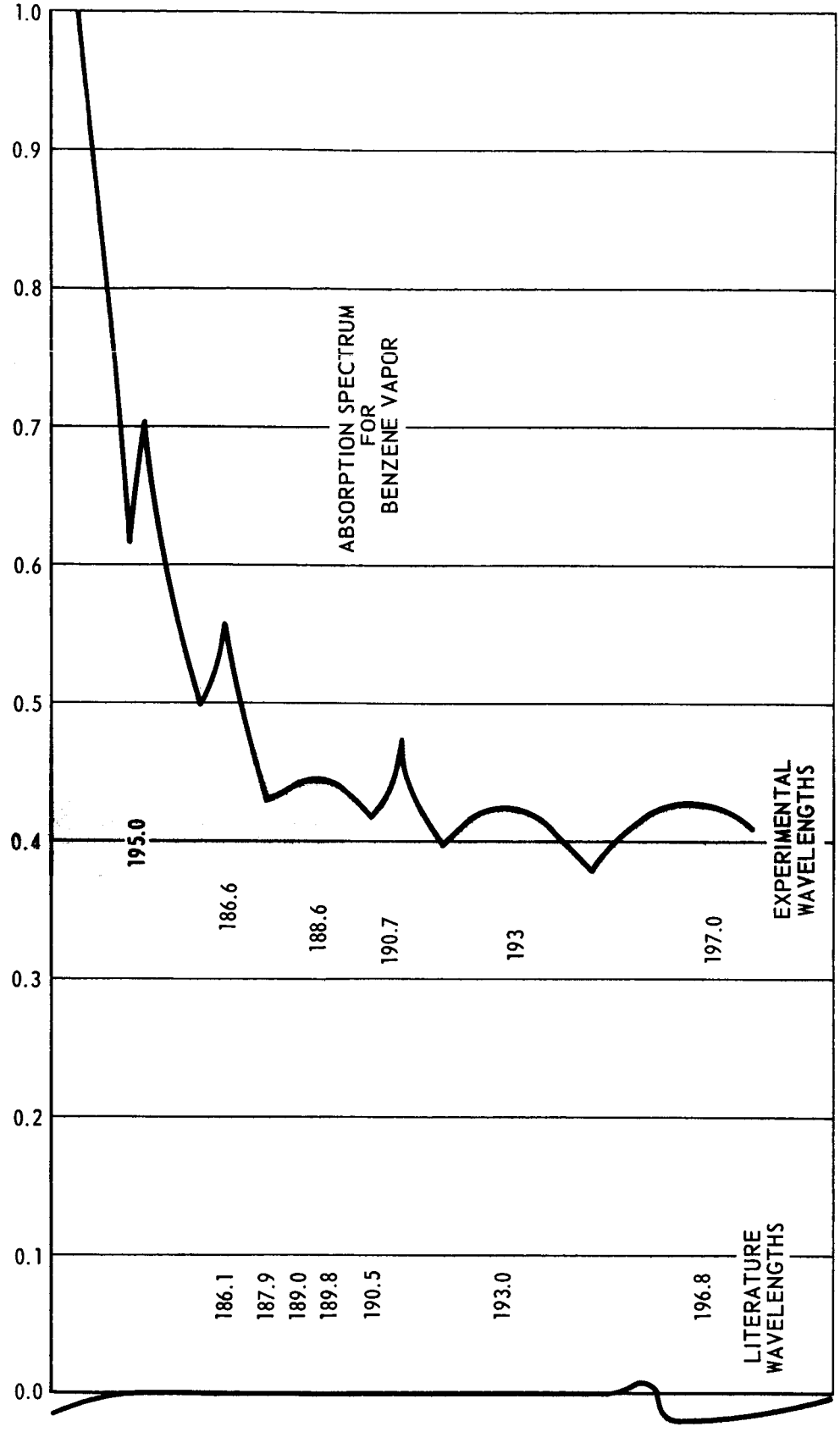

Figure 3. Calibration with Benzene Vapor

The work done during the first quarter was concerned with the solution of instrumental problems and a preliminary survey of materials of biological origin. Studies were also begun on effects of solvents, pH, and concentration.

SOLVENT EFFECT

Water is a satisfactory solvent down to a wavelength of 183 mμ. At this wavelength, the absorbance of water rises sharply, as shown in figure 4.

Dioxane, at concentrations of 50% and 25%, absorbs at wavelengths lower than 190 mμ. The effect of this absorption is to limit the use of this solvent system. It may be noted in figure 5, where the spectrum of bovine serum albumin in water and dioxane are superimposed on each other, that the dioxane has no appreciable effect on the absorption sepctrum of the protein. The absorption maximum of the protein is simply obscured by the dioxane solution.

SPECTRA OF BIOLOGICAL MATERIALS

Spectra of bovine serum all show an absorption maximum at 190 mμ, as shown in figure 6. It is noteworthy that absorbency at each maximum increases with pH until pH7. Further increase in pH results in a decrease in absorbency, as shown in figure 7. The relationship of absorbency to concentration appears to follow the Beer-Lambert law at all absorption maxima, as shown in figure 8.

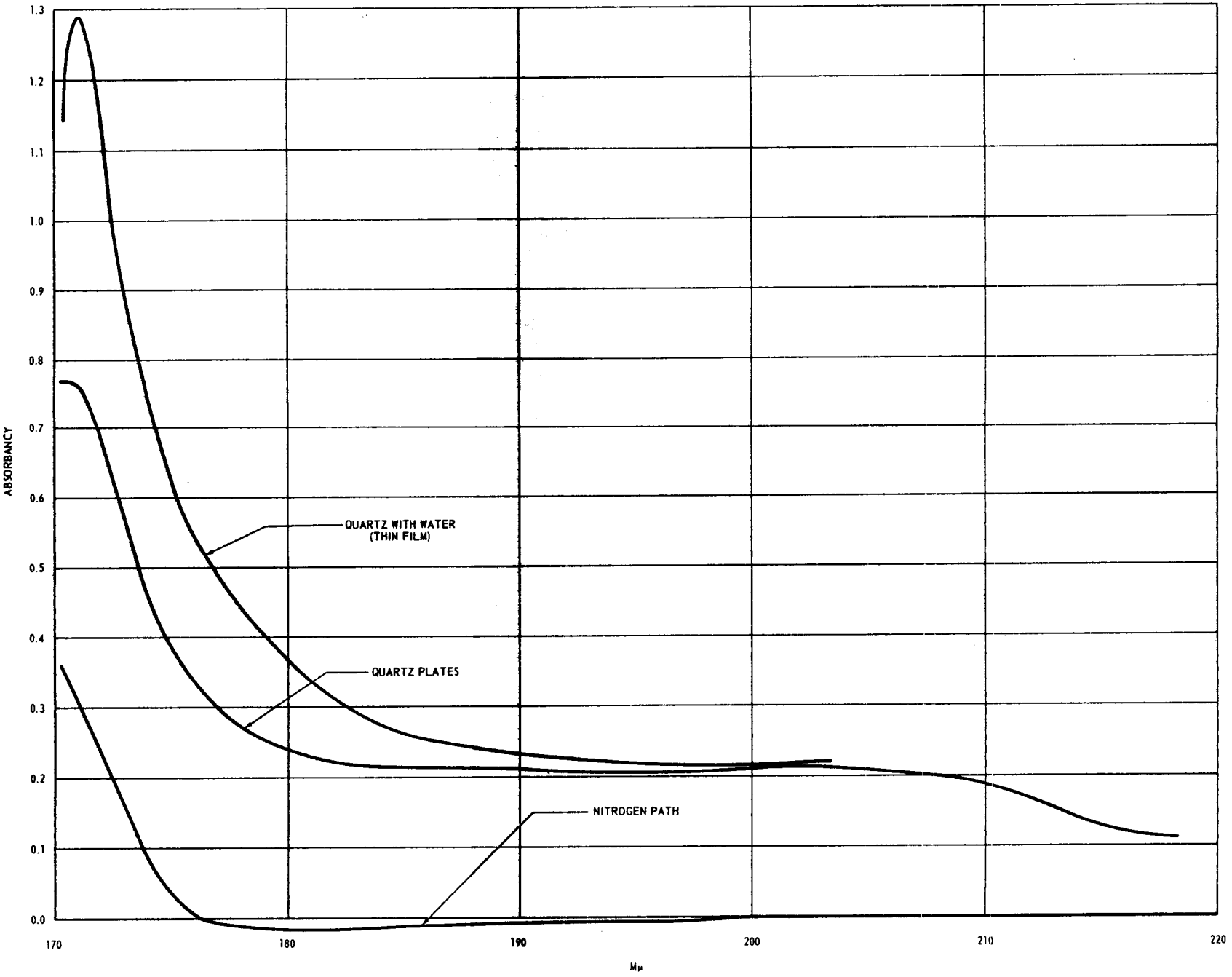

Figure 4. U. V. Absorption by Quartz and Water

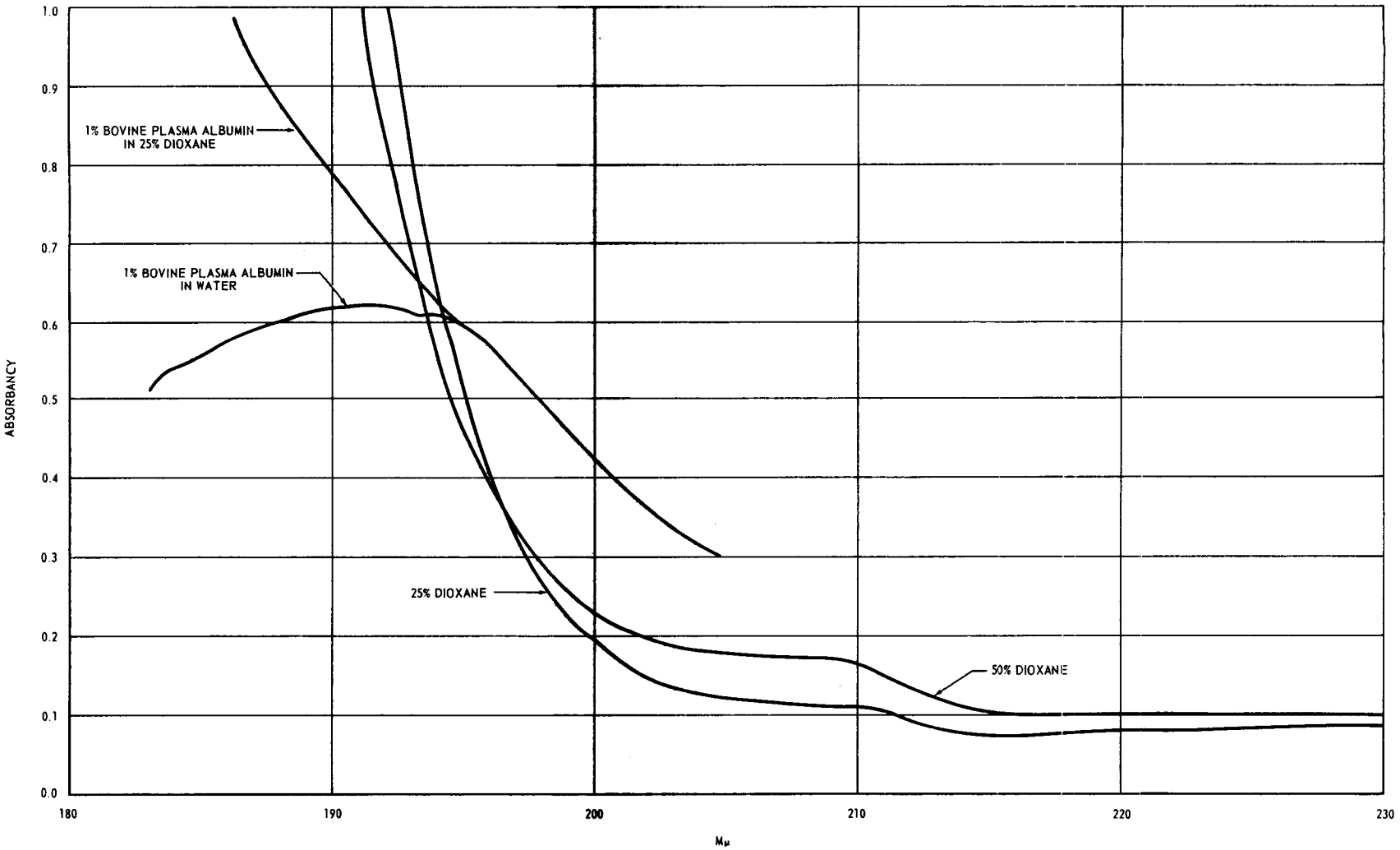

Figure 5. Solvent Effects

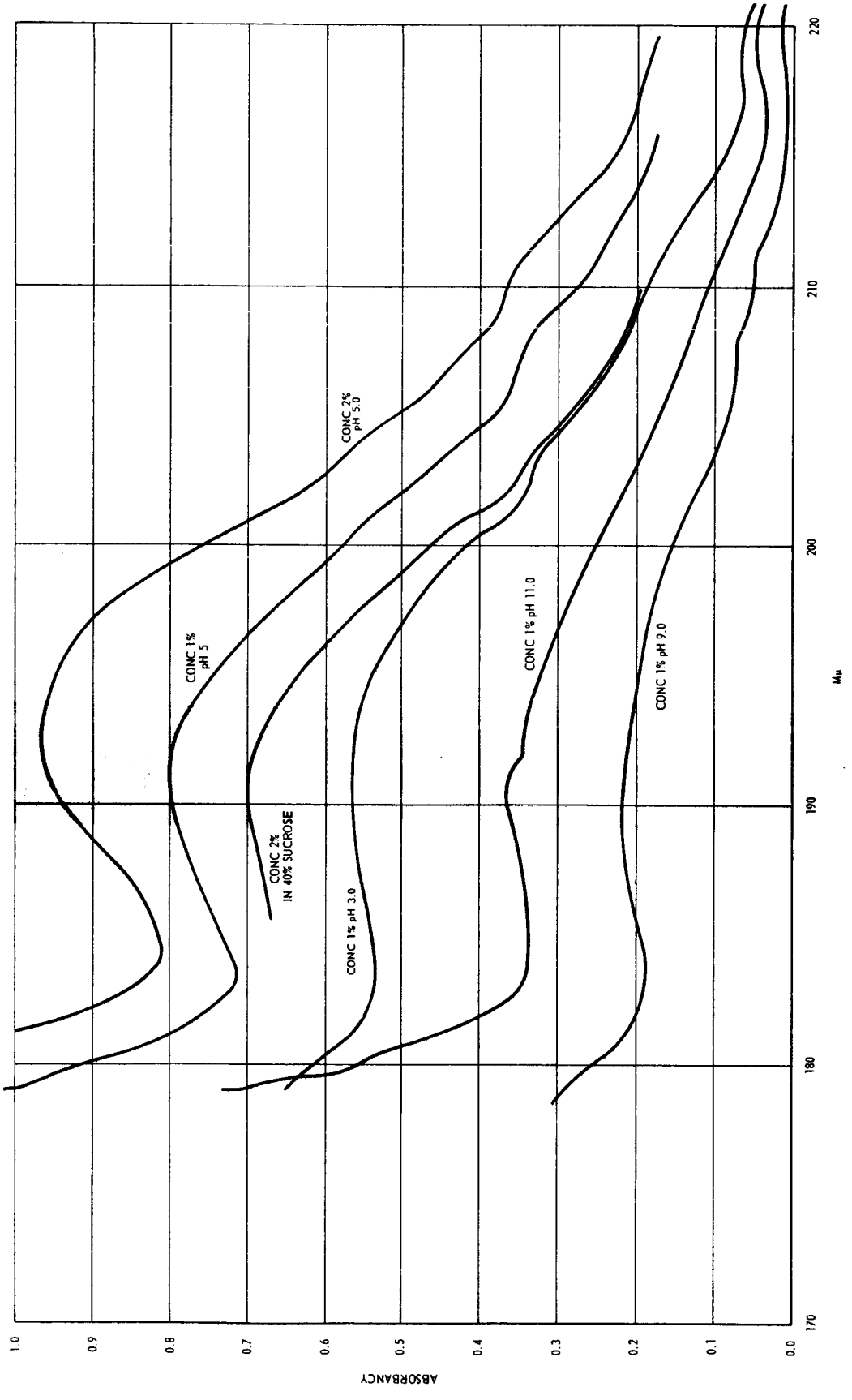

Figure 6. Absorption Spectra - Bovine Serum Albumin

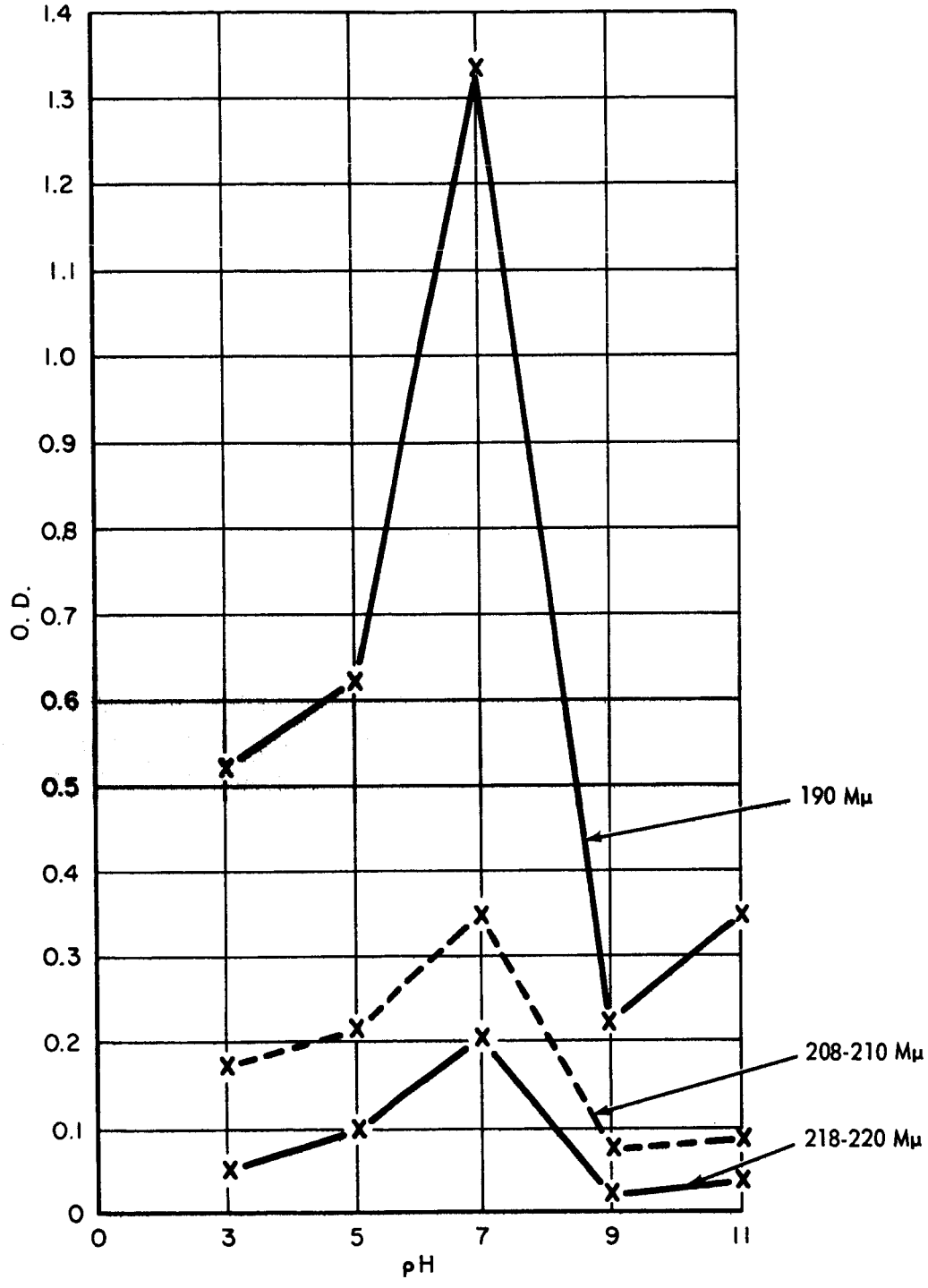

Figure 7. Effect on pH on Absorption by Bovine Serum Albumin

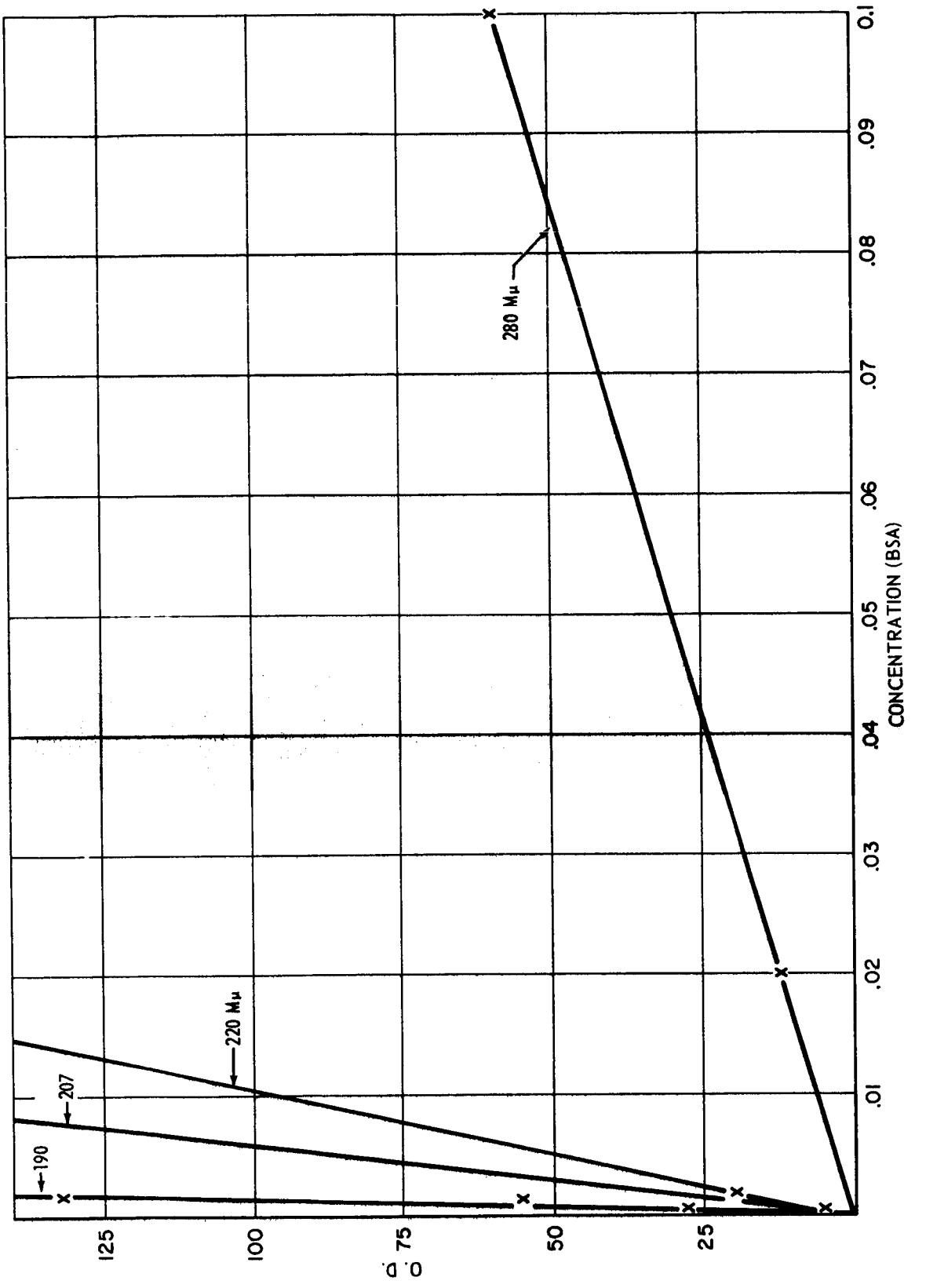

Figure 8. Beer-Lambert Plot of Bovine Serum Albumin for Four Absorption Maxima

Ribonuclease was similarly studied and was also found to have an absorption maximum at 190 mμ. Here, too, the absorbency changes with pH, as shown in figures 9 and 10.

The absorption spectra of several amino acids are shown in figure 11. It will be noted that glycine and alanine show no specific absorption at 190 mμ. On the other hand, typtophan, phenylalanine, and tyrosine all show clear maxima in this region.

Glycyl glycine is the only dipeptide which has been studied here so far. The absorption spectrum shown in figure 12 shows no maximum at 190 mμ.

Discussion

The absorption of ultraviolet light at 190 mμ by proteins has been reported as a specific absorption by the peptide bond.[1,2] Rosenheck and Doty[3] have amplified on this phenomenon and pointed out that this absorption is increased by 65% as the pH is raised and a transition from helix to random coil is brought about. Our results tend to conform generally with these reports. The results reported here, however, deviate in important details.

Thus, the absorption in question was obtained unequivocally with both bovine serum albumin and ribonuclease. An increase in absorption was noted in both cases as the pH was raised to neutrality. At pH 7.0, the absorption was at a maximum, and then fell off rapidly as the pH was raised beyond pH 7. This pH effect is in agreement with the predictions made by Rosenheck and Doty on the basis of the exciton theory.

The presence of the absorption maximum at about 190 mμ in tryptophan, phenylalanine, and tyrosine raises the question of whether the strong absorption by proteins is not caused, in part, by the presence of these amino acids. The failure to

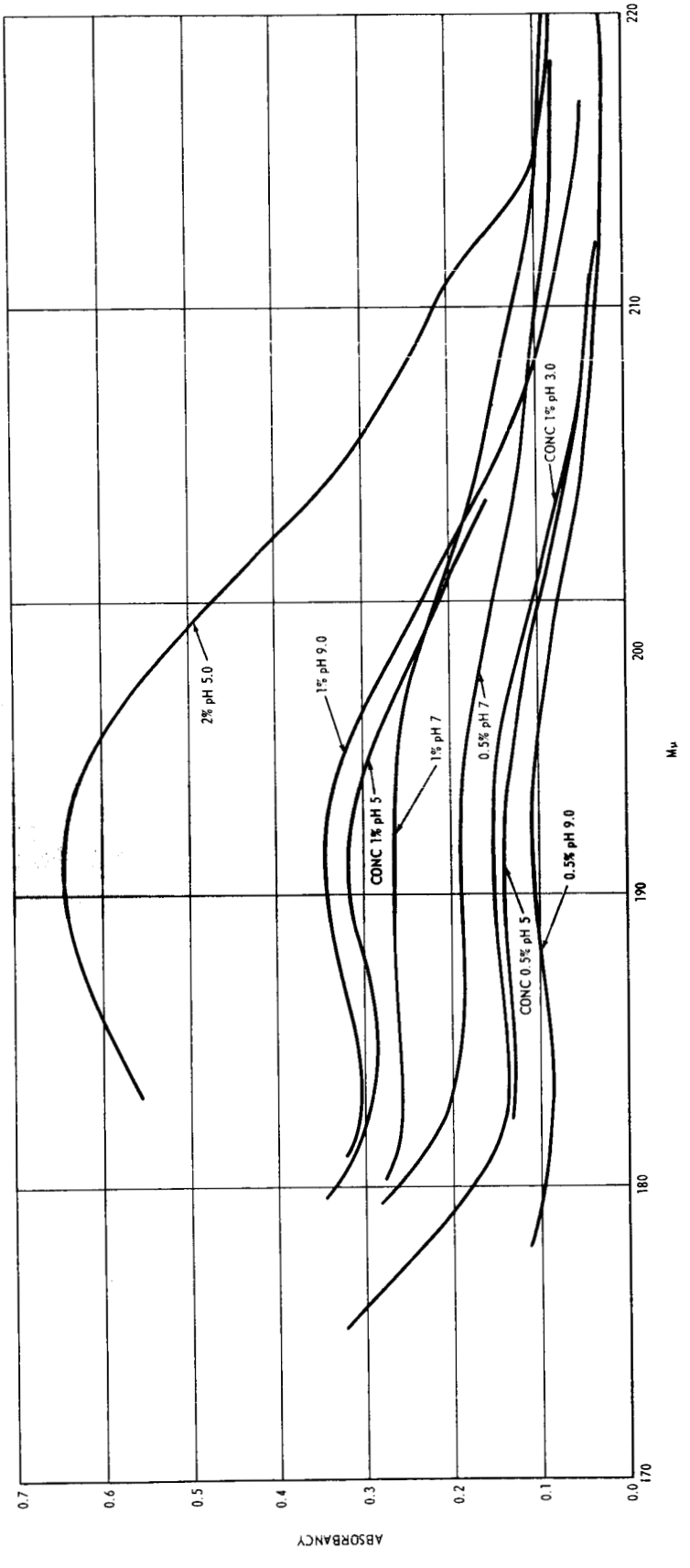

Figure 9. Absorption Spectra - Ribonuclease

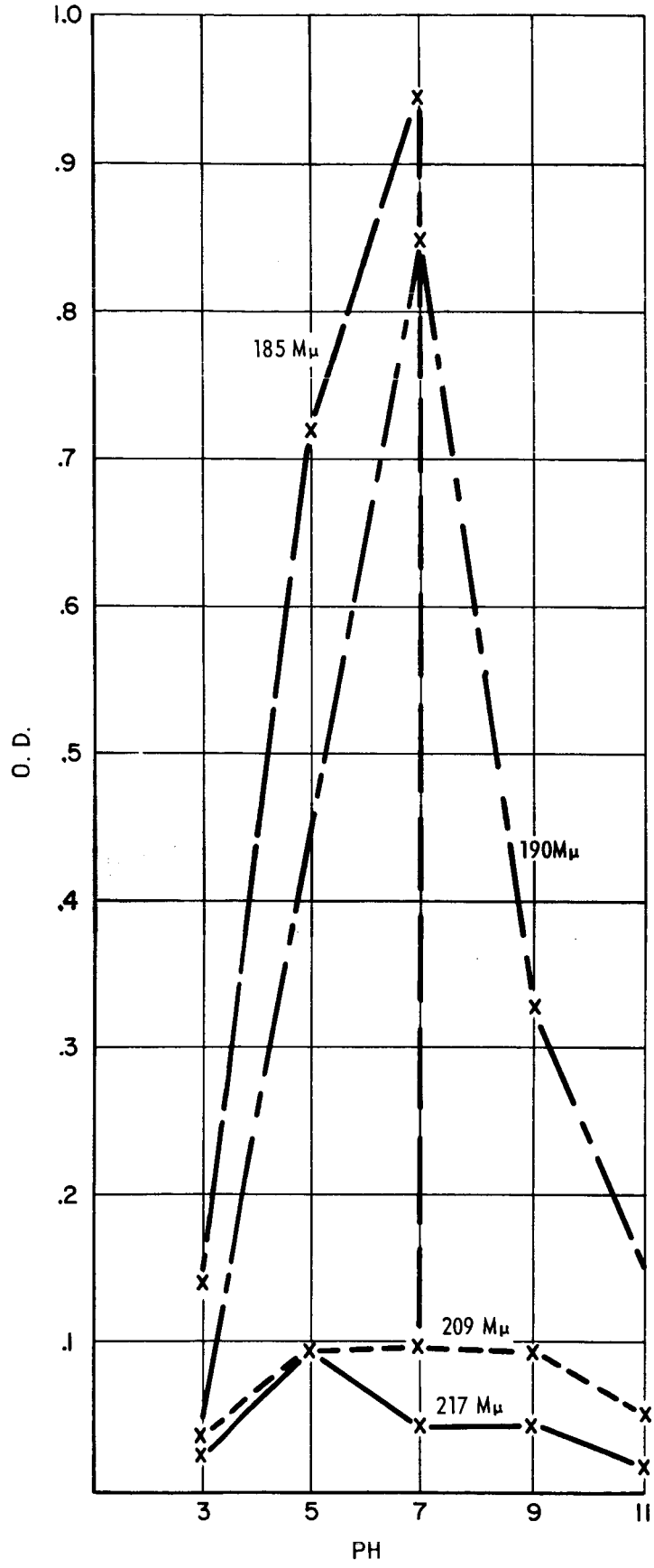

Figure 10. Effect of pH on Absorption by Ribonuclease

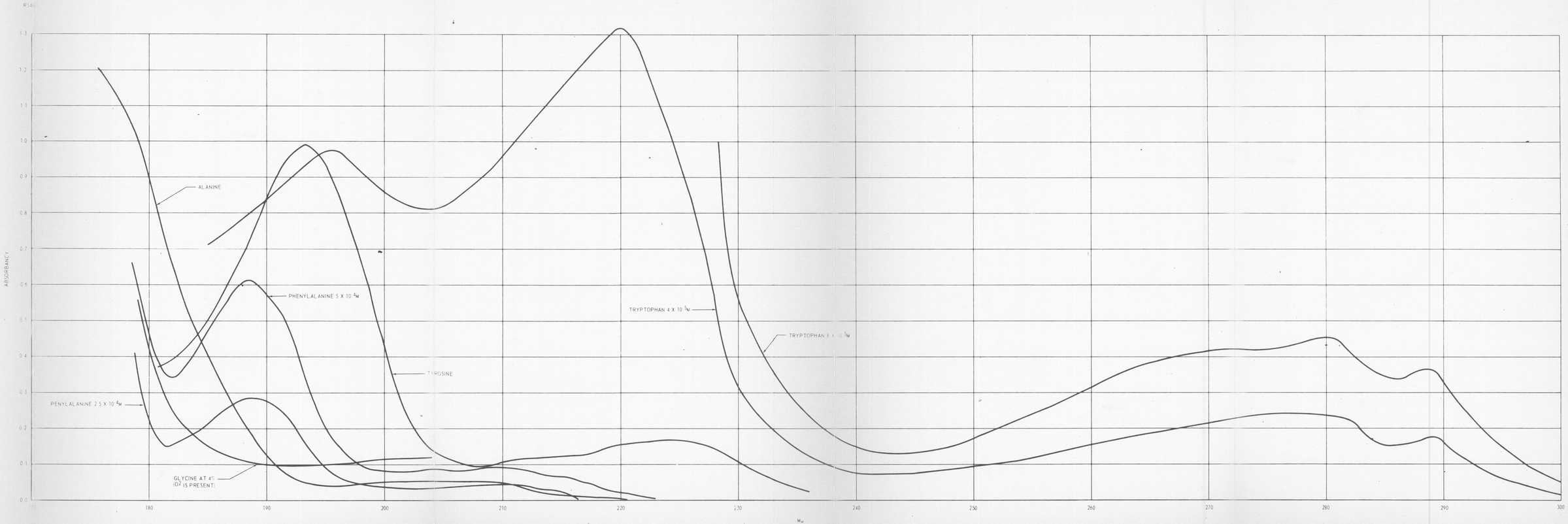

Figure 11. Absorption Spectra - Amino Acids

Figure 12. Absorption Spectrum - Glycyl Glycine

observe this absorption maximum in glycyl glycine raises the question of whether the absorption is at all characteristic of petide bonds. The observation that poly-L-lysine and poly-L-glutamic acid both show this absorption maximum[3] indicates clearly that the presence of trytophan, phenylalanine, or tyrosine is not a requisite for this absorption. On the other hand, the evidence for the specific absorption by the peptide bond is not yet conclusive, in view of its absence in glycyl glycine.

Other workers[2] have reported that glycyl glycine exhibits an absorption maximum at about 190 mμ. The published spectrum shows the peak to be at a wavelength of 185-188 mμ. The shape of the curve and its proximity to the absorption by water introduce the possibility that the observed absorption maximum may be strongly influenced by the absorption of water and its effect on the behavior of the instrument. Our own experiments with neutral density filters with absorbances equivalent to 0.5 and 1.0 show that the introduction of the latter filter causes the slits to open wide at 190 mμ. The 0.5 filter causes them to open wide at 185 mμ. Under these conditions, absorbance measurements become unreliable. The optical density of water in a thin film (See figure 4) was measured as 0.1 at 180 mμ. Because the thin film had a path length of less than 1/20 cm, the results reported by Ham and Platt[1] are questioned on the basis of the confusion of true absorption with apparent absorption caused by interference of water.

At this time, nothing conclusive can be said about this problem. The extent of the contribution of the aromatic amino acids to the absorption by the proteins can be checked by measurements on gelatin. The absorption may actually be specific

for the peptide bond, but the increase in absorption caused by the helix-random coil transition suggests the possibility that a dipeptide will not show this peak. The peptide may have to be long enough to form a helix before any strong absorption of vacuum ultraviolet becomes apparent.

Additional illuminating data might be obtained by the use of solvents that are transparent in the 180-200 mμ region. The availability of such solvents is being investigated. Meanwhile, a simpler alternative is to use thin cells and dried films.

SUMMARY

Absorption maxima between 185 and 190 mμ were found in bovine serum albumin and ribonuclease. The absorbencies at all maxima for these proteins vary with pH, go through a maximum at pH 7, and then fall off sharply. Absorption maxima between 185 and 190 mμ were found for phenylalanine, tryptophan, and tyrosine, but not for glycine, alanine, or glycyl glycine.

Present work includes a systematic study of dipeptides, tripeptides, and polypeptides, and a study of the contribution of aromatic amino acids to the observed absorption.

Among the peptides to be studied are alanyl glycyl glycine, alanyl leucine, benzoyl glycyl glycine, benzoyl glycine, glycyl glycyl glycine, leucyl glycyl glycine, leucyl tyrosine, leucyl glycine, poly lysine, and poly glutamic acid.

Following the study of the peptides, representative proteins will be examined, and then various forms of living material such as bacteria and protozoa will be studied.

15250

Further consideration will be given to the problem of instrumentation, and modification in instrumental design and procedures will be made as necessary.

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2. Priess, J. W. and Setlow, R., J. Chem. Physics, 25, 1 (1956).

3. Rosenheck, K. and Doty, P., Proc. Natl. Acad. Sci., 47, 1775 (1961).